Soil Biology and Biochemistry 124 (2018) 161–167

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Effects of artificial warming on different soil organic carbon and nitrogen pools in a subtropical plantation

Yiqing Li[a,c,1], Yanxia Qing[a,1], Maokui Lyu[a,b], Shidong Chen[a,b], Zhijie Yang[a,b], Chengfang Lin[a,b,*], Yusheng Yang[a,b]

[a] Key Laboratory for Subtropical Mountain Ecology (Ministry of Science and Technology and Fujian Province Funded), School of Geographical Science, Fujian Normal University, Fuzhou 350007, China
[b] Institute of Geography Sciences, Fujian Normal University, Fuzhou 350007, China
[c] College of Agriculture, Forestry and Natural Resources Management, University of Hawaii, Hilo, HI 96720, USA

ARTICLE INFO

Keywords:
Acid resistant C
Acid resistant N
Extracellular enzyme activity
Extractable C and N
Microbial community composition
Subtropical plantation

ABSTRACT

Uncertainty about the effects of climate warming on belowground processes of tropical and subtropical forests limits our ability to predict the response and feedback of such ecosystems to future climate change. Few field experiments in the tropics and subtropics have been conducted on the effects of manipulating warming on microbial community, enzyme activities and soil organic carbon (C) decomposition of forest ecosystems. Here, using buried cable techniques, we conducted a microcosm warming experiment to investigate extractable and acid resistant C and nitrogen (N) pools, microbial community composition, and enzyme activity after about 1.5 years of soil warming (+5 °C) in a subtropical plantation in southeastern China. The microbial community structure was quantified with phospholipid fatty acid (PLFA) analysis. Soil extractable and acid resistant C and N fractions were determined using a two-step sulfuric acid hydrolysis. We found that warming increased soil extractable C by 28% and acid resistant N decomposition by 20%. Soil warming decreased soil microbial N use efficiency by 31% but did not alter microbial C use efficiency. Warming differentially affected bacteria, fungi and enzymes activities. Our results suggest that climate warming can alter microbial community structure and enzyme activity and consequently lead to a serious imbalance between soil N and C decomposition in subtropical tree plantations.

1. Introduction

According to the RCP 8.5 scenario, global terrestrial surface temperatures will likely increase 2.6–4.8 °C by the end of this century (IPCC, 2014). Increasing temperature could have a significant impact on global carbon (C) cycles (Koven et al., 2017; Wang et al., 2015; Crowther et al., 2016). To mitigate the negative impact of global warming on terrestrial ecosystems, numerous studies have been conducted on sequestering more C into soils for a longer period of time without causing significant detrimental effects. One key factor that must be considered in a warming manipulative study is the composition and partitioning of different soil organic matter (SOM) pools with contrasting decomposability and residence time in soils. However, few studies have examined warming effects on different SOM pools and the underlying mechanisms, particularly in tropical and subtropical forest ecosystems (Cox et al., 2013; Cavaleri et al., 2015).

Measurement of the total C and nitrogen (N) of SOM does not often allow to detect their subtle changes with altered environmental factors, particularly in short-term experimental studies. Fractionating SOM into extractable and acid resistant pools based on their decomposibility and nutritional availability and quantifying these pools could be more valuable for better understanding the impact of altered environmental factors on SOM dynamics (Schmidt et al., 2011; Xu et al., 2015). For example, Zak et al. (1996) and Trumbore and Zheng (1996) found that the labile pool was very sensitive to temperature change. In contrast, Davidson and Janssens (2006) reported that acid resistant SOC was more sensitive to temperature increase with a higher Q_{10} value. Ziegler et al. (2013) and Seo et al. (2015) suggested that warming destabilized acid resistant SOC pool and caused a significant loss of soil C in temperate and boreal regions. Other studies suggested that elevated temperature decreased labile C due to enhanced microbial activity and increased acid resistant C due to C-to-N ratio changes (Cheng et al.,

* Corresponding author. Key Laboratory for Subtropical Mountain Ecology (Ministry of Science and Technology and Fujian Province Funded), School of Geographical Science, Fujian Normal University, Fuzhou 350007, China.
E-mail address: tonylcf99@fjnu.edu.cn (C. Lin).
[1] These authors made same contribution to the paper and considered to the first authors.

https://doi.org/10.1016/j.soilbio.2018.06.007
Received 27 December 2017; Received in revised form 4 June 2018; Accepted 11 June 2018
Available online 18 June 2018
0038-0717/ © 2018 Elsevier Ltd. All rights reserved.

2007; Zhou et al., 2013a). The highly inconsistent findings on the warming effects on the labile and stable fractions of SOM calls for more studies, especially in the tropical and subtropical regions with much less information available.

Microbes play a key role in soil C decomposition and N mineralization (Zhang et al., 2005; Rudrappa et al., 2006). Soil microbial biomass C and N pools are considered to be vital components of SOM in forest ecosystems and sensitive to environmental perturbation. The ratios of soil microbial biomass C (SMB-C) to extractable pool C (LP-C) and soil microbial biomass N (SMB-N) to extractable pool N (LP-N) have been used as parameters to determine nutrient use efficiency of the microbes and substrate availability in soils (Belay-Tedla et al., 2009). Warming could affect microbial community composition and SMB-C and consequently affect soil extractable C and N pools depending on site conditions (Zhou et al., 2013a, b; Garcia-Palacios et al., 2015; Tischer et al., 2015).

A variety of extracellular enzymes produced by different microbial groups, such as Gram + and Gram– bacteria, actinomycetes and mycorrhizal fungi, mediate nearly every step of SOM decomposition in different ways depending on the forms of SOM (Zumsteg et al., 2013; Cenini et al., 2016). Different forms of SOM with varying components such as cellulose, lignin, and chitin targeted by a wide range of enzymes may have different temperature sensitivities (Tischer et al., 2015). Acid resistant C sources such as wood containing much lignin and tannin were often broken down by oxidative enzymes produced by fungi and gram + bacteria, while extractable C sources were broken down mostly by hydrolytic enzymes produced by another group of bacteria (Ma et al., 2014; Xu et al., 2015). Many studies reported that C decomposition and N mineralization were closely associated with enzyme activity (e.g. Cenini et al., 2016). Hydrolytic enzymes such as β-1,4-glucosidase (βG), Cellobiohydrolase (CBH), β-1,4-N-acetylglucosaminidase (NAG) degraded mostly extractable SOC and tended to increase with mineral N concentration, while oxidative enzymes, Phenol Oxidase (PhOx) and Peroxidase (PerOx) generally degraded acid resistant SOC and were often suppressed with N additions (Acosta-Martınez et al., 2007; Cusack et al., 2011; Wallenstein and Burns, 2011; Ma et al., 2014). Although previous studies demonstrated that interactions between microbial resource demands and SOM quality determined the direction and magnitude of soil C responses to temperature change (e.g., Billings and Ballentyne, 2013) and SOM-decaying enzymes changes with temperature (Billings et al., 2016), data was scarce on the interactions of enzymes and decomposition of various SOM fractions under field experimental warming conditions, especially in the tropics and subtropics.

The dominant species of China plantations is Chinese fir (*Cunninghamia lanceolata*), which accounts for most commercial plantations with respect to acreage and timber production (Lu et al., 2014). We used Chinese fir planting as a model system to understand the C and N response to the effects of artificial soil warming by conducting a heating cable approach. A thorough understanding of the responses of extractable and acid resistant C and N of Chinese fir plantation to warming is critical for accurately predicting belowground C dynamics of subtropical plantations with future climate change.

In the present study, by using soil warming manipulation, C and N fractionation and phospholipid fatty acid (PLFA) techniques, we examined the effects of soil warming on different C and N pools, soil microbial community structure, enzyme activities and SOC decomposition rates in a subtropical young plantation. Specifically, we aimed to answer the following questions: (1) How will soil warming affect extractable and acid resistant soil organic C and N decomposition in a Chinese fir plantation? and (2) How will soil microbial community structure and enzyme activity change with soil warming and consequently affect the processes of soil C and N decomposition?

2. Materials and methods

2.1. Site and experimental design

This research was conducted at Saming experimental site of the Research Station of Forest Ecosystems and Global Change of Fujian Province, South China (26°19 N, 117°36 E). The study area has a typical subtropical monsoon climate with a mean annual temperature of 19.1 °C, a mean annual precipitation of 1670 mm, and an average humidity of 80%. The mean annual precipitation is approximately 1656 mm, > 70% of the precipitation occurring from March to August. The mean annual air temperature is 19.1 °C and the relative humidity averages 80%. The soil is classified as red soil based on the China's soil classification systems, equivalent to Oxisols in the USDA Soil Taxonomy. Overstory tree species are dominated by *castanopsis carlesii*, *Castanopsis fissa*, *Schima superba*, *Lithocarpus glaber*, *Symplocos caudate*, *Machilus velatina*. During the past several decades, the majority of the broadleaf evergreen natural forests in this region were converted to plantations of Chinese fir (*Cunninghamia lanceolata*) to meet the growing demand for timber, fuel material, and other non-timber forest products (Guo et al., 2016). The total area of the Chinese fir plantations was over 9.11 million ha. The present study was carried out in a young Chinese fir plantation established in 2013.

The experiment included five warming and five control plots. Each plot had a size of 2 × 2 m. The experiment was established in August 2013. Heating cables (TXLP/1, Nexans, Norway) were buried in both control and warming soils at a depth of 10 cm with a horizontal interval of 20 cm but the cables in the control plots were not heated. Data were not collected after 6 months to minimize the difference between the disturbed and undisturbed controls. Temperature sensors (T109, Campbell Scientific Inc. Logan, USA) were placed between cables at a depth of 10 cm, three in each warming plot and two in each control plot. Soil temperature in the warming plots was continuously maintained at 5 °C above the temperature in the control plots. A datalogger maintained this differential temperature by switching the cables on and off on a 2-min cycle.

2.2. Soil sampling

In November 2015, soil was sampled with a 3.5-cm soil sampler to a depth of 0–10 cm. Six cores were randomly taken in each plot. All soil samples were immediately transferred to the laboratory and stored in a refrigerator at 4 °C until chemical analyses occurred within a week. Subsamples for soil water content were oven-dried at 105 °C for 24 h. Subsamples for analysis of soil pH, total organic C and N, and inorganic N were air-dried for 2–4 days, ground and passed through a 2-mm sieve. Subsamples for PLFA and enzyme analyses were stored at a temperature of −20 °C. Soil pH was determined using a pH meter at a soil:water ratio of 1:2.5. Total soil C and N were determined by using an elemental analyzer (Elementar Vario MAX, EA Consumables, Inc. USA). For nitrate and ammonium analyses, five grams of fresh soil from each sample were extracted with a 2-mol L^{-1} KCl solution. The extractant was shaken for 40 min and then filtered for nitrate and ammonium determination using a Continuous Flow Analyzer (SKALAR san++, Holland).

2.3. Enzyme analysis

Enzyme analysis was conducted by following a procedure as described in Saiya-Cork et al. (2002) and Sinsabaugh et al. (1992). Enzymes assayed in this study included acid phosphatase, beta glucosidase, cellobiohydrolase, β-1,4-N-acetylglucosaminidase, phenol oxidase and peroxidase. Suspensions of 1 g soil to 125 ml of acetate buffer at a concentration of 50 mol L^{-1} were prepared for each sample and agitated for 1 min using a Brinkmann Polytron PT 3000 homogenizer. The sample suspensions were continuously mixed with a magnetic stir plate

during which 200 ml of the suspensions was portioned into 96-well microplates at 16 replicate wells per sample per assay. Fluorimetric assays were conducted for all enzymes except phenol oxidase and peroxidase. Fluorescence was measured using a microplate fluorometer with 365 nm excitation and 450 nm emission filter. After a correction for quenching and negative controls all activity units were presented as nmol h^{-1} g^{-1}, 4-dihydroxyphenylalanine (DOPA) was used as the substrate for spectrophotometrical analysis for phenol oxidase and peroxidase activity. Each sample well had 50-μl solution of 25 mM DOPA and peroxidase assays had 10-μl of 0.3% H_2O_2. Blank wells had 200-μl of the sample suspension and 50 μl of acetate buffer. A 50-μl solution of DOPA and 200 μl of acetate buffer were added to negative control wells for phenol oxidase. Negative control and blank wells for peroxidase had 10 μl of H_2O_2. This produced 8 replicate wells for blanks and controls, and 16 replicate sample wells for each enzyme assay. Microplates were incubated at 20 °C for 18 h in the dark. Activity was considered as a measure of absorbance at 450 nm by using a microplate spectrophotometer.

2.4. PLFA analysis

The microbial community structure was determined using the phospholipid fatty acid (PLFA) analysis as described by White et al. (1979) and Bardgett et al. (1996). In summary an extracted mixture of chloroform, methanol and citrate buffer with a volume ratio of 1:2:0.8 from a 10-g dry sieved soil was used for PLFA analysis. The extraction process included two phases: the chloroform phase and the citrate buffer phase. The lipid materials were recovered and evaporated under nitrogen gas during the chloroform phase. These lipids were then re-suspended in chloroform and then fractionated on silicic chromatography acid columns (CNWBOND, 500 mg, 3 ml). Neutral lipids, glycolipids and phospholipids were eluted with 5 ml of chloroform, acetone and methanol respectively. Nitrogen gas was used to dry the phospholipid fraction before a mild alkaline methanolysis was conducted to prepare for fatty acid methyl esters. For alkaline methanolysis, one ml of methyl alcohol and methylbenzene with a ratio of 1:1 in volume and then 1.0 ml of methanolic KOH were added to each sample. After swirling to mix, the samples were sealed and placed in bath at 37 °C for 30 min. Afterwards, 2.0 ml of hexane was added to each sample and after swirling 0.2 ml of 1.0 M acetic acid was added to each sample. Two ml of deionized H_2O was added to each sample to break phase and vortex samples for 30 s and then the samples were centrifuged for 2 min. The top phase was transferred to clean labeled 10-ml vials with a short Pasteur pipette. Afterwards, 2.0 ml of hexane was added to each sample and then swirled. Nitrogen gas was also used to evaporate these methyl esters and stored at −20 °C until running gas chromatography analysis (GC). For the CG process, 200-μl HPLC-grade ethyl acetate was used to dissolve and separate the individual methyl esters. They were then run on GC with SGE 25QC3 BP-5 25 m × 0.32 μm film in thickness. A chromographic retention time comparison to bacterial methyl esters was used to identify and quantify the separated fatty acids (Supeloc Bacterial Acid Methyl Esters CP Mix 47080-U). Relative ηmoles per gram of dry soil was used to express the abundance of individual fatty acids using a standard nomenclature (Tunlid et al., 1989; Frostegård et al., 1993a, b). PLFAs that represented gram-positive bacteria (GP) were considered to be i-14:0, i-15:0, a-15:0, i-16:0, i-17:0, and a-17:0, and the PLFAs that represented gram-negative bacteria (GN) were 16:1 w9c, 16:1 w7c, cy-17:0 w7c, 18:1 w7c, cy-19:0 w7c. PLFAs that represented fungal species (fungi) were 18:2 w6c and 18:1 w9c. PLFAs for actinomycetes (ACT) were 16:0 10-methyl, 17:0 10-methyl, 18:0 10-methyl, 17:1 w7c 10-methyl, and 18:1 w7c 10-methyl. The PLFA for arbuscular mycorrhizae and (VAM) anaerobes (DMA) were 16:1 w5c and 15:0 respectively. For the total bacteria (Tbacteria) determination the PLFAs for gram + and gram- 14:0, 15:0, 17:0 and 18:0 were included. The ratio of fungal to bacterial PLFAs (F:B) was used to estimate of the relative importance of the bacterial and fungal metabolic presence in the community.

2.5. Carbon and nitrogen pools analysis

To determine extractable and acid resistant concentrations of SOC and SON, 20 ml of 5N H_2SO_4 was added to 500 mg dry soil, and the sample was hydrolyzed for 30 min at 105 °C in a teflon tube. The hydrolysate was centrifuged for 20 min and then passed through a 0.45-μm filter paper. This initially extracted hydrolysate included extractable pool I carbon (LPI-C) and extractable pool I nitrogen (LPI-N). The residue from this process was flushed repeatedly with deionized water and then oven-dried at 60 °C. The samples were hydrolyzed with 2 ml of 13 mol L^{-1} H_2SO_4. The samples were continuously shaken for at least 10 h at room temperature. Acid concentration was reduced to 1 mol L^{-1} with de-ionized water and then hydrolyzed at 105 °C for 3 h. The hydrolysate was then centrifuged at 4500 r min^{-1} and passed through a 0.45-μm filter paper. This hydrolysate included extractable pool II carbon (LPII-C) and nitrogen (LPII-N). Remaining residue was taken out and washed with deionized water repeatedly and then oven-dried at 60 °C. Acid resistant pool carbon (RP-C) and nitrogen (RP-N) were determined using a C-N analyzer (ElementarVario, MAX, Germany).

2.6. Microbial biomass C and N

Soil microbial biomass carbon (SMB-C) and nitrogen (SMB-N) were determined using the chloroform ($CHCl_3$) fumigation and potassium sulfate (K_2SO_4) extraction techniques as described by Vance et al. (1987) and Xu et al. (2015). In summary, $CHCl_3$ was used to fumigate 5-g fresh soil for 24 h in dark plastic bags paired with unfumigated controls. A 20-ml solution of 0.5 mol L^{-1} K_2SO_4 were used and the samples were fumigated and then shaken for 30 min at a rate of 250 r min^{-1}. Samples were centrifuged for 10 min at 4000 r min^{-1} and then passed through a 0.45-μm glass fabric filter paper.

2.7. Dissolved organic C and N, ammonium and mineral nitrogen

Dissolved organic carbon (DOC) and nitrogen (DON) was extracted with deionized water. Ten grams of dry sieved soil and 40 ml of deionized water were used to make a solution at a ratio of 1:4 and then was shaken for 30 min at 250 r min^{-1}. Samples were centrifuged for 10 min at a rate of 4000 r min^{-1} and then passed through a 0.45-μm glass fabric filter paper.

Soil ammonium (NH_4^+) and nitrate nitrogen (NO_3^-) was extracted using potassium chloride (KCl). A 5-g soil sample was added to 20 ml of KCl at a concentration of 2 mol L^{-1} to form a mixture which was shaken for 30 min at 250 r min^{-1}, centrifuged for 10 min at a rate of 4000 r min^{-1} and then filtered using a 0.45-μm filter paper. The extractants were then used for NH_4^+ and NO_3^- analysis.

2.8. Soil pH, SOC and SON

Soil pH was determined using 10-g dry soil and 25 ml CO_2-free water at a soil:water ratio of 1:2.5. The mixture was stirred for 20 min and then settled for 30 min at room temperature. pH was measured using a pH meter. The dry sieved soil was analyzed for SOC and SON using a C-N analyzer (ElementarVario, MAX, Germany).

2.9. Statistical analysis

All statistical analyses were done using the IBM SPSS Statistics 22 version 19.0 software (SPSS Inc. Chicago, IL), OriginPro 9.0 (OriginLab Corporation, Northampton, Mass) and CANOCO 5.0 software (Microcomputer Power Inc. Ithaca, NY). An independent-sample *t*-test was used to determine the effects of warming on soil microbial enzyme concentrations, PLFA biomarkers, LPI-C, LPII-C, RP-C, LPI-N, LPII-N, and RP-N values, MBC, MBN, DON, DOC, SOC, SON, pH, ammonium,

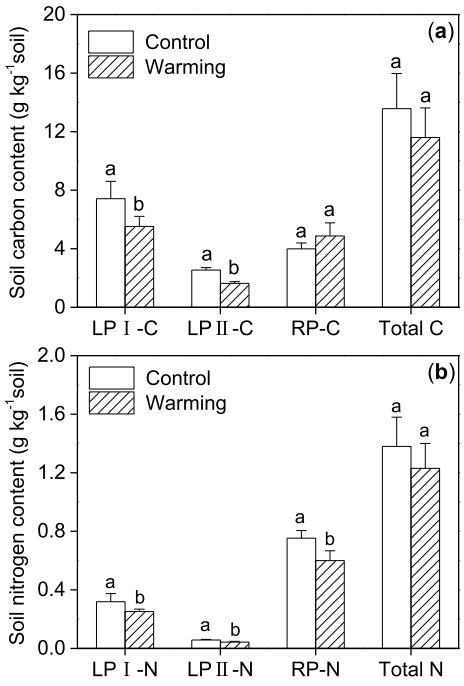

Fig. 1. Amount of C and N pool fractions, including (a) labile C pool I (LPI-C, mainly polysaccharides) and II (LPII-C, mostly cellulose), and recalcitrant C pool (RP-C), and total organic C pool sizes, and (b) labile N pool I (LPI-N, mainly polysaccharides) and II (LPII-N, mostly cellulose), and recalcitrant N pool (RP-N), and total organic N pool sizes in control and warmed plots. Values are mean ± SD ($n = 5$). Different letters between control and warming treatment means significant difference at $P < 0.05$ level.

and nitrate. Analysis of similarities (ANOSIM) based on Bray-Curtis distances was used to test the significance of microbial communities between the treatments (Oksanen et al., 2007). Redundancy analysis (RDA) was used to test the treatment effects on soil microbial community structure and environmental variables.

3. Results

3.1. Extractable and acid resistant pools of soil C and N

Soil warming did not affect the size of total organic C pool but significantly reduced extractable C pool I (LPI-C) and pool II (LPII-C) in the top 10 cm of the soils (Fig. 1a). The content of LPI-C was 5.53 (± 0.69) and 7.42 (± 1.18) g kg^{-1} soil for the warming treatment and the control respectively and the content of LPII-C was 1.64 (± 0.11) and 2.55 (± 0.16) g kg^{-1} soil for the warming and the control respectively. We did not find a significant difference in acid resistant C pool (RP-C) between the warming treatment and the control though there was an increasing trend in the warming treatment (Fig. 1a). The content of RP-C was 4.88 (± 0.89) and 3.99 (± 0.40) g kg^{-1} soil for the warming and control, respectively.

There was a significant difference in all N pools with lower values for the three pools in the warming treatment (Fig. 1b). The content of extractable N pool I (LPI-N) was 0.25 (± 0.016) and 0.32 (± 0.056) g kg^{-1} soil for the warming and the control respectively. The content of extractable N pool II (LPII-N) was 0.043 (± 0.006) and 0.057 (± 0.004) g kg^{-1} soil for the warming and the control respectively. The content of acid resistant N pool (RP-N) was 0.599 (± 0.067) and 0.753 (± 0.052) g kg^{-1} soil for the warming and the control, respectively (Fig. 1b).

Table 1
Effects of soil warming on soil microbial biomass carbon (SMB-C), nitrogen (SMB-N), soil microbial biomass C:N ratio (SMB-C:SMB-N); dissolved organic C (DOC); dissolved organic N (DON); nitrate nitrogen (NO_3^--N); ammonium nitrogen (NH_4^+-N) and soil pH. The values are means ± SD ($n = 5$). Bold font means significant differences at $p < 0.05$ level.

	Control	Warming	Independent *t*-test t_8 (*P*-value)
SMB-C (mg kg^{-1})	295.24 ± 59.98	212.71 ± 12.76	$t_8 = -3.01$ (**0.017**)
SMB-N (mg kg^{-1})	24.99 ± 5.19	15.21 ± 1.46	$t_8 = -4.05$ (**0.004**)
SMB-C:SMB-N	12.11 ± 2.99	14.09 ± 1.54	$t_8 = 1.31$ (0.226)
DOC (mg kg^{-1})	4.96 ± 1.28	6.98 ± 0.83	$t_8 = 2.96$ (**0.018**)
DON (mg kg^{-1})	7.52 ± 0.56	6.49 ± 0.49	$t_8 = -3.09$ (**0.015**)
NH_4^+-N (mg kg^{-1})	1.94 ± 0.64	2.08 ± 0.62	$t_8 = 0.35$ (0.739)
NO_3^--N (mg kg^{-1})	4.19 ± 0.39	6.57 ± 1.07	$t_8 = 4.68$ (**0.002**)
Soil pH	4.53 ± 0.10	4.66 ± 0.12	$t_8 = 1.86$ (0.101)

3.2. DOC, DON and soil microbial C and N

Soil warming significantly ($p < 0.05$) reduced soil microbial biomass C (SMB-C) and microbial biomass N (SMB-N) (Table 1). Warming did not significantly affect the ratio of SMB-C:SMB-N, soil NH_4^+-N and soil pH. Warming increased soil dissolved organic C (DOC) and NO_3^--N but decreased dissolved organic N (DON) (Table 1).

The ratio of SMB to extractable pool (LP) for both C and N was also altered by warming (Fig. 2). Warming significantly decreased the SMB-N:LPN ratio ($p < 0.05$) but did not significantly affect the SMB-C:LPC.

3.3. Soil enzyme activities and microbial PLFAs

Warming altered both the C- and N-degrading enzyme activities (Table 2). Warming increased the C-degrading enzymes βG ($p < 0.01$) and CBH ($p < 0.01$) but decreased PHO ($p < 0.01$). Warming decreased N-degrading enzyme NAG ($p < 0.001$) but had no significant effect on P-degrading enzyme AP (Table 2). In addition, the ratio of N-degrading to P-degrading enzymes was significant lower in the warming treatment than the control ($t_4 = -4.24$, $p = 0.003$; data not shown).

Warming significantly affected the microbial community composition (Fig. 3). All the PLFA biomarkers showed a decreasing trend. The PLFA of total microbes, total bacteria, gram-negative bacteria, unspecific bacteria, actinomycete, fungus and anaerobe significantly ($p < 0.05$) decreased with warming compared to the control. However, warming did not significantly affect the PLFA of gram-positive bacteria and arbuscular mycorrhizal fungi although there was a

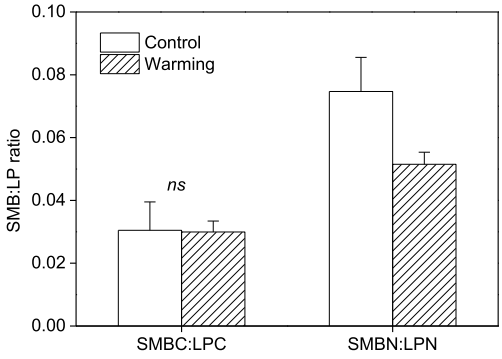

Fig. 2. Soil microbial biomass to labile pool ratio (SMBC: LPC and SMBN: LPN) under experimental warming. Values are mean ± SD ($n = 5$). *ns*, no significance; *, $P < 0.05$.

Table 2
Effects of soil warming on soil enzyme activity. βG: β-1, 4-glucosidase, CBH: Cellobiohydrolase; PHO: Phenol Oxidase; PE: Peroxidase; NAG: β-1, 4-N-acetylglucosaminidase; AP: Acid phosphatase. The values are means ± SD (n = 5). Bold font means significant differences at $p < 0.05$ level.

Treatment	C-degrading Enzymes				N-degrading enzyme	P-degrading enzyme
	βG	CBH	PHO	PE	NAG	AP
Control	10.3 ± 2.7	3.3 ± 0.8	4.4 ± 0.5	26.4 ± 3.4	315.8 ± 60.7	68.2 ± 11.7
Warming	26.6 ± 5.5	5.5 ± 1.1	3.1 ± 0.5	25.3 ± 2.1	159.5 ± 23.0	58.4 ± 13.4
Paired t-test	$t_8 = 5.91$	$t_8 = 3.59$	$t_8 = -4.45$	$t_8 = -0.61$	$t_8 = -5.39$	$t_8 = -1.23$
p-values	p = **0.000**	p = **0.007**	p = **0.002**	p = 0.557	p = **0.001**	p = 0.254

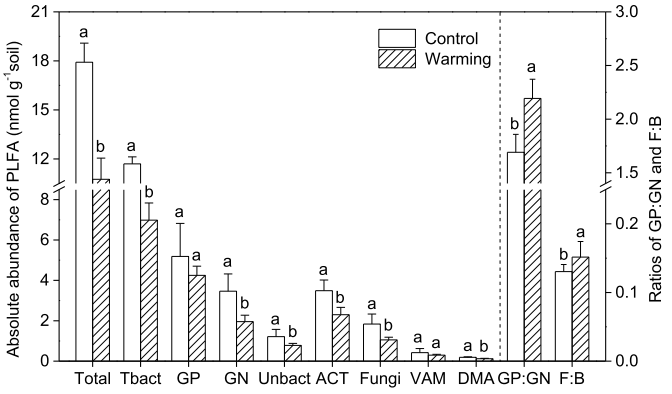
Absolute abundance of PLFA (nmol g-1 soil)
Ratios of GP:GN and F:B
Control
Warming
Total Tbact GP GN Unbact ACT Fungi VAM DMA GP:GN F:B

Fig. 3. Effects of soil warming on the phospholipid fatty acid biomarker contents (in nmol g^{-1} soil). Total, total microbial PLFAs; fungi, total fungi PLFAs; Tbact, total bacteria PLFAs, the sum of GP, GN and unspecific bacteria; B: F, the ratio of total bacteria to total fungi PLFAs; GP, total gram-positive bacteria PLFAs; GN, total gram-negative bacteria PLFAs; GP: GN, the ratio of total gram-positive bacteria to gram-negative bacteria PLFAs. VAM, Arbuscular mycorrhiza; ACT: actinomycetes; DMA: anaerobe; unbact: unspecific bacteria. Values are mean ± SD (n = 5). Different letters between control and warming treatment means significant difference at $P < 0.05$ level; the marginal significance at $P < 0.1$.

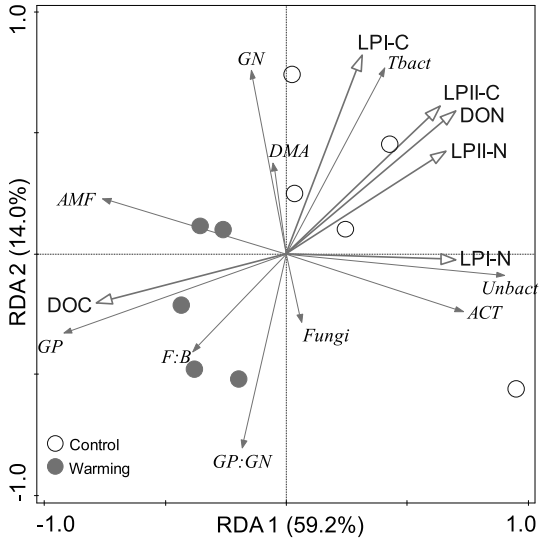

Fig. 4. Redundancy analysis ordination biplot of PLFA profiles indicating the relationships between the variation of microbial community composition and environmental parameters [labile carbon pool I (LPI-C), labile carbon pool II (LPII-C), recalcitrant carbon pool (RP-C), labile nitrogen pool I (LPI-N), labile nitrogen pool II (LPII-N), recalcitrant nitrogen pool (RP-N), microbial biomass carbon (MBC), Cellobiohydrolase (CBH) and Phenol Oxidase (PHO)]; Tbact, total bacteria PLFAs, B:F, the ratio of total bacteria to total fungi PLFAs; GP, total gram-positive bacteria PLFAs; GN, total gram-negative bacteria PLFAs; GP:GN, the ratio of total gram-positive bacteria to gram-negative bacteria PLFAs. VAM, Arbuscular mycorrhiza; ACT: actinomycetes; DMA: anaerobe; unbact: unspecific bacteria.

decreasing trend in the warming plots (Fig. 3). Warming significantly increased the ratio of gram-positive bacteria to gram-negative bacteria and marginally significantly increased the ratio of fungi to bacteria (Fig. 3).

3.4. Correlation between soil PLFAs and physicochemical variables

Based on redundancy analysis (RDA), there was a significant difference in microbial community composition between the warming treatment and the control (ANOSIM, $R = 0.636$, $p = 0.005$). Warming explained 59.9% of the variance (Fig. 4). The RDA showed that DOC, DON and LPII-C accounted for 37.4%, 34.1% and 29.8% of the variance respectively (Table 3). Soil microbial community composition was significantly correlated with LPI-C and LPII-N, which explained 30.6 and 28.8% of the variance respectively ($p < 0.1$). The first axis was positively correlated with DON, LPII-N, LPI-N and LPII-C but negatively correlated with DOC. The second axis was positively correlated with LPI-C and all variables explained 73.2% of the variance of microbial composition in the warming treatment (59.2%) and the control (14.0%) (Fig. 4).

Table 3
Correlations between environment parameters and RDA ordination of % mol abundance of PLFAs in control and warming treatments. Significant differences are indicated by bold-face p values and the italic indicates marginal significance.

Environment parameters	Explains %	pseudo-F	p
DOC	37.4	4.8	**0.026**
DON	34.1	4.1	**0.024**
LPII-C	29.8	3.4	**0.022**
LPI-C	30.6	3.5	*0.052*
LPII-N	28.8	3.2	*0.052*
LPI-N	16.5	1.6	0.230

4. Discussion

Prediction of climate change largely depends on effects of warming on SOM decomposition and the understanding of temperature sensitivities of different SOM fractions (Davidson and Janssens, 2006; Luo, 2007; Belay-Tedla et al., 2009). This is especially so for tropical forests, which accounts for a third of global soil C and remains an important source of uncertainty in climate model projections (Jobbagy and Jackson, 2000; Cox et al., 2013; Cavaleri et al., 2015). Belay-Tedla et al. (2009) reported that warming increased extractable C content in soils due to warming-induced increase of above- and below-ground biomass. Several long-term studies reported that extractable soil C was positively correlated with the litter input (Cambardella and Elliott, 1992; Janzen et al., 1992; Wieder et al., 2015). However, Peterjohn et al. (1993) and Xu et al. (2015) found that labile pool size of SOM decreased with warming as a result of increased soil respiration and SOM decomposition. In our study we found that warming significantly reduced

extractable C pool I (LPI-C) and pool II (LPII-C) in the top 10 cm of the soils. This finding indicates that the onset of warming increases the extractable C decomposition rate and thus reduces extractable C in the soils with little litter input at the early stage of the plantation.

Previous studies reported that the decomposition of extractable C rather than acid resistant C may be accelerated under a warming condition (Giardina and Ryan, 2000; Bradford et al., 2008; Xu et al., 2010, 2015). Our result that short-term warming did not significantly change soil acid resistant C but had an increasing trend indicates that long-term warming may increase acid resistant C in subtropical tree plantations.

Previous studies found that warming could increase net N mineralization and nitrification rate by 52.2 and 32.2% respectively (Bai et al., 2013), leading to N loss from ecosystems. Dawes et al. (2016) and Melillo et al. (2011) reported that experimental soil warming in temperate forests stimulated soil organic N mineralization. We found in the present study that soil warming reduced both extractable and acid resistant organic N pools but increased mineral N, which indicates warming may enhance mineralization of organic N in subtropical forest soils.

Our result that both extractable C and N were reduced in the warming treatment implies that warming enhances soil microbial activity in the subtropical plantation. In the present study SMB-C and SMB-N were significantly reduced in the warming plots compared to control (Table 1). Soil warming might lead to higher (Belay-Tedla et al., 2009), lower (Frey et al., 2008) or unvarying (Biasi et al., 2008) microbial biomass. Microbial C use efficiency has long been the focus of many studies in soil biogeochemistry, while microbial N use efficiency refers to the partitioning of organic N taken up between growth and the release of inorganic N to the environment (that is, N mineralization), has relatively less been studied (Mooshammer et al., 2014). SMB/LP ratio can be considered as a measure of nutrient use efficiency by microbes (Belay-Tedla et al., 2009). The finding in the present study that warming decreased SMB-N/LP-N ratios but did not change SMB-C/LP-C (Fig. 2) could not be explained with the data obtained in this experiment. Probably multifactorial experiments with both N and warming as treatments are needed in such forest plantations.

Soil microorganisms drive soil biogeochemical processes and soil microbial community directly affects organic matter degradation and its response to temperature change (Ostle and Ward, 2012). An important variable in determining how warming will affect SOC decomposition is the relative dominance of bacteria and fungi. Fungi are associated with a slow energy channel (slow turnover of more acid resistant and N-poor substrates, leading to high soil-C accumulation), whereas bacteria are associated with a fast energy channel (fast turnover of extractable and N-rich substrates), leading to low soil-C accumulation (Clemmensen et al., 2013; Bardgett and van der Putten, 2014). Our result that warming significantly increased the ratio of gram-positive bacteria to gram-negative bacteria and fungal to bacteria (Fig. 3) implies a possible shift from bacterial-to fungal-dominated microbial communities. This change may be associated with a reduction in SOC as found in previous studies (Clemmensen et al., 2013; Bardgett and van der Putten, 2014). The RDA analysis showed that the variations in the soil microbial community structure were associated with extractable C and N pools and dissolved organic C and N in the soils. This finding suggests that warming may affect the soil microbial community structure by mediating the quantity of microbial substrates and the availability of C and N. The DOC concentration was negatively correlated with the first axis, indicating an association with the gram-positive bacteria and fungi biomass, which supported the previous study that DOC in soils was a product of decomposition process (Schimel and Bennett, 2004). The increase in DOC and decrease in DON under warming likely resulted from the lower N use efficiency under warming treatment as suggested by Weintraub et al. (2007), Chen and Xu (2008) and Carrera et al. (2015). Therefore, a potential shift in the soil microbial community structure could primarily be regulated by soil substrate availability and nutrient use efficiency under climate warming.

Our study showed that warming stimulated the extractable C-degrading enzymes (β-glucosidase and cellobiohydrolase) but not the acid resistant C-degrading enzymes (phenol oxidase and peroxidase) activities. This result could be a direct explanation for the decrease in soil extractable C pools. The change in enzyme activity can be attributed to the changes in microbial community structure as well as its sensitivity to warming. The lower ratio of N-degrading to P-degrading enzymes in this study implies lower N use efficiency with warming treatment as reported by Nottingham et al. (2015) and Cenini et al. (2016). The result on enzyme activity in the present study indicates that warming can differentially affect the extractable C- and acid resistant C-degrading enzymes and consequently change the SOC decomposition and sequestration. This field warming experiment conducted in China is one of pioneer studies in the subtropics. However, due to limited resource (high demand for power) and topography constraints we used 2×2 measuring plots, which is too small for the research results to be extrapolated to the entire subtropical forest ecosystems.

In conclusion, soil warming enhanced extractable SOC decomposition and did not change acid resistant SOC. Warming reduced extractable and acid resistant N and soil microbial N use efficiency. Fungal and bacterial phospholipid fatty acid biomarker content significantly decreased under warming condition. Extractable C-degrading enzymes were significantly increased with warming treatment. Our results suggest that climate warming can remarkably alter microbial community structure and enzyme activity and thus consequently lead to a serious imbalance between soil N and C decomposition in subtropical forests.

Acknowledgements

This study was jointly supported by the National Key Basic Research Program of China (2014CB954003) and the National Natural Science Foundation of China (No. U1405231 and No. 31130013). We thank Drs. Christian Giardina and Francis Bowles for help in experimental design and warming system control.

References

Acosta-Martınez, V., Cruz, L., Sotomayor-Ramırez, D., Perez-Alegrıa, L., 2007. Enzyme activities as affected by soil properties and land use in a tropical watershed. Applied Soil Ecology 35, 35–45.

Bai, E., Li, S., Xu, W., Li, W., Dai, W., Jiang, P., 2013. A meta-analysis of experimental warming effects on terrestrial nitrogen pools and dynamics. New Phytologist 199, 441–451.

Bardgett, R.D., Hobbs, P.J., Frostegård, Å., 1996. Soil fungal:bacterial ratios following reductions in the intensity of management of an upland grassland. Biology and Fertility of Soils 22, 261–264.

Bardgett, R.D., van der Putten, W.H., 2014. Belowground biodiversity and ecosystem functioning. Nature 515, 505–511.

Belay-Tedla, A., Zhou, X.H., Su, B., Wan, S.Q., Luo, Y.Q., 2009. Labile, recalcitrant, and microbial carbon and nitrogen pools of a tallgrass prairie soil in the US Great Plains subjected to experimental warming and clipping. Soil Biology and Biochemistry 41, 110–116.

Biasi, C., Meyer, H., Rusalimova, O., Hammerle, R., Kaiser, C., Baranyi, C., Daims, H., Lashchinsky, N., Barsukov, P., Richter, A., 2008. Initial effects of experimental warming on carbon exchange rates, plant growth and microbial dynamics of a lichen-rich dwarf shrub tundra in Siberia. Plant and Soil 307, 191–205.

Billings, S.A., Ballantyne, F., 2013. How interactions between microbial resource demands, soil organic matter stoichiometry and substrate reactivity determine the direction and magnitude of soil respiratory responses to warming. Global Change Biology 19, 90–102.

Billings, S., Min, K., Ballantyne IV, F., Chen, Y., Sellers, M., 2016. Aging exo-enzymes can create temporally shifting, temperature-dependent resource landscapes for microbes. Biogeochemistry 131, 163–172.

Bradford, M.A., Davies, C.A., Frey, S.D., Maddox, T.R., Melillo, J.M., Mohan, J.E., Reynolds, J.F., Treseder, K.K., Wallenstein, M.D., 2008. Thermal adaptation of soil microbial respiration to elevated temperature. Ecology Letters 11, 1316–1327.

Cambardella, C.A., Elliott, E.T., 1992. Particulate soil organic matter changes across a grassland cultivation sequence. Soil Science Society of America Journal 56, 777–783.

Carrera, N., van Bodegom, P.M., van Hal, J.R., Logtestijn, R., Barreal, M.E., Briones, M.J.I., 2015. Disentangling thermal acclimation and substrate limitation effects on C and N cycling in peatlands. Soil Biology and Biochemistry 90, 224–231.

Cavaleri, M.A., Reed, S.C., Smith, W.K., Wood, T.E., 2015. Urgent need for warming experiments in tropical forests. Global Change Biology 21, 2111–2121.

Cenini, V.L., Fornara, D.A., McMullan, G., Ternan, N., Carolan, R., Crawley, M.J., Clement, J.C., Lavorel, S., 2016. Linkages between extracellular enzyme activities and the carbon and nitrogen content of grassland soils. Soil Biology and Biochemistry 96, 198–206.
Chen, C.R., Xu, Z.H., 2008. Analysis and behavior of soluble organic nitrogen in forest soils. Journal of Soils and Sediments 8, 363–378.
Cheng, L., Leavitt, S.W., Kimball, B.A., Pinter, P.J., Ottman, M.J., Matthias, A., Wall, G.W., Brooks, T., Williams, D.G., Thompson, T.L., 2007. Dynamics of labile and recalcitrant soil carbon pools in a sorghum free-air CO_2 enrichment (FACE) agroecosystem. Soil Biology and Biochemistry 29, 2250–2263.
Clemmensen, K.E., Bahr, A., Ovaskainen, O., Dahlberg, A., Ekblad, A., Wallander, H., Stenlid, J., Finlay, R.D., Wardle, D.A., Lindahl, B.D., 2013. Roots and associated fungi drive long-term carbon sequestration in boreal forest. Science 339, 1615–1618.
Cox, P.M., Pearson, D., Booth, B.B., Friedlingstein, P., Huntingford, C., Jones, C.D., Luke, C.M., 2013. Sensitivity of tropical carbon to climate change constrained by carbon dioxide variability. Nature 494, 341–344.
Crowther, T.W., Todd-Brown, K.E.O., Rowe, C.W., Wieder, W.R., Carey, J.C., Machmuller, M.B., Snoek, B.L., Fang, S., Zhou, G., Allison, S.D., Blair, S.J.M., Bridgham, D., Burton, A.J., Carrillo, Y., Reich, P.B., Clark, J.S., Classen, A.T., Dijkstra, F.A., Elberling, B., Emmett, B.A., Estiarte, M., Frey, S.D., Guo, J., Harte, J., Jiang, L., Johnson, B.R., Kröel-Dulay, G., Larsen, K.S., Laudon, H., Lavallee, J.M., Luo, Y., Lupascu, M., Ma, L.N., Marhan, S., Michelsen, A., Mohan, J., Niu, S., Pendall, E., Peñuelas, J., Pfeifer-Meister, L., Poll, C., Reinsch, S., Reynolds, L.L., Schmidt, I.K., Sistla, S., Sokol, N.W., Templer, P.H., Treseder, K.K., Welker, J.M., Bradford, M.A., 2016. Quantifying global soil carbon losses in response to warming. Nature 540 (7631), 104–108.
Cusack, D.F., Silver, W.L., Torn, M.S., Burton, S.D., Firestone, M.K., 2011. Changes in microbial community characteristics and soil organic matter with nitrogen additions in two tropical forests. Ecology 92 (3), 621–632.
Davidson, E.A., Janssens, I.A., 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440, 165–173.
Dawes, M.A., Schleppi, P., Hättenschwiler, S., Rixen, C., Hagedorn, F., 2016. Soil warming opens the nitrogen cycle at the alpine treeline. Global Change Biology. http://dx.doi.org/10.1111/gcb.13365.
Frey, S.D., Drijber, R., Smith, H., Melillo, J., 2008. Microbial biomass, functional capacity, and community structure after 12 years of soil warming. Soil Biology and Biochemistry 40, 2904–2907.
Frostegård, A., Bååth, E., Tunlid, A., 1993a. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. Soil Biology and Biochemistry 25, 723–730.
Frostegård, A., Tunlid, A., Bååth, E., 1993b. Phospholipid fatty acid composition, biomass, and activity of microbial communities from two soils types experimentally exposed to different heavy metals. Applied and Environmental Microbiology 59, 3605–3617.
Garcia-Palacios, P., Vandegehuchte, M.L., Shaw, E.A., Dam, M., Post, K.H., Ramirez, K.S., Sylvain, Z.A., De Tomasel, C.M., Wall, D.H., 2015. Are there links between responses of soil microbes and ecosystem functioning to elevated CO_2, N deposition and warming? A global perspective. Global Change Biology 21, 1590–1600.
Giardina, A., Ryan, M., 2000. Evidence that decomposition rates of organic carbon in mineral soil do not vary with temperature. Nature 404, 858–861.
Guo, J.F., Yang, Z.J., Lin, C.F., Liu, X.F., Chen, G.S., Yang, Y.S., 2016. Conversion of a natural evergreen broadleaved forest into coniferous plantations in a subtropical area: effects on composition of soil microbial communities and soil respiration. Biology and Fertility of Soils 52 (6), 799–809.
IPCC, 2014. In: Core Writing Team, Pachauri, R.K., Meyer, L.A. (Eds.), Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC, Geneva, Switzerland, pp. 151.
Janzen, H.H., Campbell, C.A., Brandt, S.A., Lafond, G.P., Townley-Smith, L., 1992. Light fraction organic matter in soils from long-term crop rotations. Soil Science Society of America Journal 56, 1799–1806.
Jobbagy, E.G., Jackson, R.B., 2000. The vertical distribution of soil organic carbon and its relation to climate and vegetation. Ecological Applications 10, 423–436.
Koven, C.D., Hugelius, G., Lawrence, D.M., Wieder, W.R., 2017. Higher climatological temperature sensitivity of soil carbon in cold than warm climates. Nature Climate Change 7, 7817–7822.
Lu, S., Wang, Q., Katahara, S., Naramoto, M., Mizunaga, H., 2014. Soil microbial activities in beech forests under natural incubation conditions as affected by global warming. Pedosphere 24, 709–721.
Luo, Y., 2007. Terrestrial carbon cycle feedback to climate warming. Annual Review of Ecology, Evolution and Systematics 38, 683–712.
Ma, Y., Zhu, B., Sun, Z., Zhao, C., Yang, Y., Piao, S., 2014. The effects of simulated nitrogen deposition on extracellular enzyme activities of litter and soil among different-aged stands of larch. Journal of Plant Ecology 7, 240–249.
Melillo, J.M., Butler, S., Johnson, J., Mohan, J., Steudler, P., Lux, H., Burrows, E., Bowles, F., Smith, R., Scott, L., Vario, C., Hill, T., Burton, A., Zhou, Y.M., Tang, J., 2011. Soil warming, carbon–nitrogen interactions, and forest carbon budgets. Proceedings of the National Academy of Sciences 108, 9508–9512.
Mooshammer, M., Wanek, W., Hämmerle, I., Fuchslueger, L., Hofhansl, F., Knoltsch, A., Schnecker, J., Takriti, M., Watzka, M., Wild, B., Keiblinger, K.M., Zechmeister-Boltenstern, S., Richter, A., 2014. Adjustment of microbial nitrogen use efficiency to carbon:nitrogen imbalances regulates soil nitrogen cycling. Nature Communications 5, 3694. http://dx.doi.org/10.1038/ncomms4694.
Nottingham, A.T., Turner, B.L., Whitaker, J., Ostle, N., McNamara, N.P., Bardgett, R.D., Salinas, N., Meir, P., 2015. Soil microbial nutrient constraints along a tropical forest elevation gradient: a belowground test of a biogeochemical paradigm. Biogeosciences Discussions 12, 6489–6523.
Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Stevens, M.H.H., Oksanen, M.J., Suggests, M., 2007. The vegan package. Community ecology package 10.
Ostle, N., Ward, S., 2012. Climate change and soil biotic carbon cycling. In: Wall, D.H. (Ed.), Soil Ecology and Ecosystem Services. Oxford University Press, pp. 241–255.
Peterjohn, W.T., Melillo, J.M., Bowles, S.T., 1993. Soil warming and trace gas fluxes: experimental design and preliminary flux results. Oecologia 93, 18–24.
Rudrappa, L., Purakayestha, T.J., Singh, D., Bhadraray, S., 2006. Long-term manuring and fertilization effects on soil organic carbon pools in a Typic Haplustept of semi-arid sub-tropical India. Soil Tillage Research 88, 180–192.
Saiya-Cork, K.R., Sinsabaugh, R.L., Zak, D.R., 2002. Effects of long term nitrogen deposition on extracellular enzyme activity in an Acer saccharum forest soil. Soil Biology and Biochemistry 34, 1309–1315.
Schimel, J.P., Bennett, J., 2004. Nitrogen mineralization: challenges of a changing paradigm. Ecology 85, 591–602.
Seo, J., Jang, I., Young, J., Kyung Lee, Y., Kang, H., 2015. Warming and increased precipitation enhance phenol oxidase activity in soil while warming induces drought stress in vegetation of an Arctic ecosystem. Geoderma 259, 347–353.
Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., K€ogel-Knabner, I., Lehmann, J., Manning, D.A.C., Nannipieri, P., Rasse, D.P., Weiner, S., Trumboreet, S.E., 2011. Persistence of soil organic matter as an ecosystem property. Nature 478, 49–56.
Sinsabaugh, R.L., Antibus, R.K., Linkins, A.E., McClaugherty, C.A., Rayburn, L., Repert, D.A., Weiland, T., 1992. Wood decomposition over a first-order watershed: mass loss as a function of lignocellulase activity. Soil Biology and Biochemistry 24, 743–749.
Tischer, A., Blagodatskaya, E., Hamer, U., 2015. Microbial community structure and resource availability drive the catalytic efficiency of soil enzymes under land-use change conditions. Soil Biology and Biochemistry 89, 226–237.
Trumbore, S.E., Zheng, S.H., 1996. Comparison of fractionation methods for soil organic matter C-14 analysis. Radiocarbon 38, 219–229.
Tunlid, A., Hoitink, H.A.J., Low, C., White, D.C., 1989. Characterization of bacteria that suppress Rhizoctonia damping-off in bark compost media by analysis of fatty acid biomarkers. Applied and Environmental Microbiology 55, 1368–1374.
Vance, E., Brookes, P., Jenkinson, D., 1987. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry 19 (6), 703–707.
Wallenstein, M.D., Burns, R.G., 2011. Ecology of extracellular enzyme activities and organic matter degradation in soil: a complex community-driven process. In: Dick, R.P. (Ed.), Methods of Soil Enzymology. Soil Science Society of America, Madison, WI, pp. 35–48 SSSA Book Ser. 9.
Wang, X., Liu, L.L., Piao, S.L., Janssens, I.A., Tang, J.W., Liu, W.X., Chi, Y.G., Wang, J., Xu, S., 2015. Soil respiration under climate warming: differential response of heterotrophic and autotrophic respiration. Global Change Biology 20, 3229–3237.
Weintraub, M.N., Scott-Denton, L.E., Schmidt, S.K., Monson, R.K., 2007. The effects of tree rhizodeposition on soil exoenzyme activity, dissolved organic carbon, and nutrient availability in a subalpine forest ecosystem. Oecologia 154, 327–338.
White, D.C., Davis, W.M., Nickels, J.S., King, J.D., Bobbie, R.J., 1979. Determination of the sedimentary microbial biomass by extractible lipid phosphate. Oecologia 40, 51–62.
Wieder, W.R., Cleveland, C.C., Smith, W.K., Todd-Brown, K., 2015. Future productivity and carbon storage limited by terrestrial nutrient availability. Nature Geoscience 8, 441–444.
Xu, G., Chen, J., Berninger, F., Pumpanen, J., Bai, J., Yu, L., Duan, B., 2015. Labile, recalcitrant, microbial carbon and nitrogen and the microbial community composition at two Abies faxoniana forest elevations under elevated temperatures. Soil Biology and Biochemistry 91, 1–13.
Xu, Z., Wan, C., Xiong, P., Tang, Z., Hu, R., Cao, G., Liu, Q., 2010. Initial responses of soil CO_2 efflux and C, N pools to experimental warming in two contrasting forest ecosystems, Eastern Tibetan Plateau, China. Plant and Soil 336, 183–195.
Zhang, W., Parker, K.M., Luo, Y., Wan, S., Wallace, L.L., Hu, S., 2005. Soil microbial responses to experimental warming and clipping in a tallgrass prairie. Global Change Biology 11, 266–277.
Zhou, X., Chen, C., Wang, Y., Xu, Z., Duan, J., Hao, Y., Smaill, S., 2013a. Soil extractable carbon and nitrogen, microbial biomass and microbial metabolic activity in response to warming and increased precipitation in a semiarid Inner Mongolian grassland. Geoderma 206, 24–31.
Zhou, X.Q., Chen, C.R., Wang, Y.F., Xu, Z.H., Han, H.Y., Li, L.H., Wan, S.Q., 2013b. Warming and increased precipitation have differential effects on soil extracellular enzyme activities in a temperate grassland. The Science of the Total Environment 444, 552–558.
Ziegler, S.E., Billings, S.A., Lane, C.S., Li, J., Fogel, M.L., 2013. Warming alters routing of labile and slower-turnover carbon through distinct microbial groups in boreal forest organic soils. Soil Biology and Biochemistry 60, 23–32.
Zak, D.R., Ringelberg, D.B., Pregitzer, K.S., Randelett, D.L., White, D.C., Curtis, P.S., 1996. Soil microbial communities beneath *Populus grandidentata* grown under elevated atmospheric CO_2. Ecological Applications 6, 257–262.
Zumsteg, A., Bååth, E., Stierli, B., Zeyer, J., Frey, B., 2013. Bacterial and fungal community responses to reciprocal soil transfer along a temperature and soil moisture gradient in a glacier forefield. Soil Biology and Biochemistry 61, 121–132.